

### **REMARKS**

The present application relates to inbred maize plant and seed PH0GC. Claims 1-30 are pending in the present application. No new matter has been added by way of amendment. Applicant respectfully requests consideration of the claims in view of the following remarks.

#### **Detailed Action**

Applicant acknowledges that because this application is eligible for continued examination under 37 C.F.R. § 1.114 and the fee set forth in 37 C.F.R. § 1.17(e) has been timely paid, the finality of the previous Office Action has been withdrawn pursuant to 37 C.F.R. § 1.114. Applicant further acknowledges that Applicant's submission filed on October 21, 2005 has been entered.

#### **Request for Information under 37 C.F.R. § 1.105**

The Examiner has made a Request for Information under 37 C.F.R. § 1.105. The Examiner states the requested information is "required to make a meaningful and complete search of the prior art". See Office Action, pp. 2-3 and 13-15.

Applicant provides answers to each of the Examiner's interrogatories discussed *infra*. Applicant notes that the information provided to the third and fourth interrogatories are only to *previously* publicly disclosed or sold parental maize lines or progeny therefrom as requested by the Examiner. Thus, Applicant asserts the interrogatories have been answered with respect to the Examiner's request for the information for prior art purposes. Applicant points out that the third interrogatory was specific to *previously* publicly disclosed or sold as this is relevant to the Examiner's prior art inquiry. Thus Applicant notes that the response to the fourth interrogatory is also answered with respect to maize lines produced by said method using said original parental maize lines which were *previously* publicly disclosed, sold or disclosed in a U.S. patent application as this is relevant to the Examiner's request for prior art purposes as stated on page 13 of the Office Action.

The Examiner begins by asking firstly, what were the original parental maize lines used to produce maize inbred line PH0GC? PH0GC was derived from a synthetic population named SYN92F.

Secondly, what method and steps were used to produce maize inbred line PH0GC? Pedigree selection method produced from SYN92F by selfing and ear rowing from F0 through F11 generation.

Third, have any of said parental maize lines (a) or progeny (b) therefrom been previously publicly disclosed or sold?

a. Pioneer Hi-Bred has not previously publicly disclosed or sold the synthetic SYN92F.

b. Pioneer Hi-Bred has not previously publicly disclosed or sold progeny of the synthetic SYN92F.

Fourth, were any other maize lines produced by said method using said original parental maize lines, and if so, have said produced maize lines been publicly disclosed, sold or disclosed in a U.S. patent application? If so, under what designation were said other maize lines disclosed or sold? No maize line using the synthetic SYN92F has been previously publicly disclosed, sold or disclosed in a U.S. patent application. Further, the question has been answered with respect to previously publicly disclosed or sold lines.

In light of the above remarks, Applicant respectfully requests reconsideration and compliance with the interrogatories under the Request for Information under 37 C.F.R. § 1.105.

#### **Rejections Under 35 U.S.C. § 112, Second Paragraph**

Claims 11-30 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. *See Office Action*, pp. 3-4.

The Examiner states claim 11 is indefinite "because it is unclear what the metes and bounds of a maize plant having all of the physiological and morphological characteristics of inbred maize line PH0GC are."

Applicant traverses this rejection. Claim 11 specifically claims a maize plant having all the physiological and morphological characteristics of inbred line PH0GC. Claim 11 encompasses maize plants having the characteristics of inbred line PH0GC. Applicant believes the Examiner is making the assumption that the fact that one must use seed of the maize inbred line PH0GC itself to obtain a plant with the same morphological and physiological characteristics as a plant of the variety PH0GC. However, one of ordinary skill in the art can obtain a plant with all of the same morphological and physiological characteristics as maize

inbred line PH0GC without actually using seed of maize inbred line PH0GC. For example, this can be accomplished by using double haploid technology to "recreate" PH0GC through the use of F1 hybrid seed in which PH0GC was a parent. As emphasized in previous office action responses, all members of the genus of F1 hybrids seed will receive one non-recombinant set of chromosomes of PH0GC. By using the seed of an F1 hybrid made with PH0GC, one can recover this non-recombined set of chromosomes from the F1 hybrid seed. Thus, a plant that has all of the same morphological and physical characteristics of PH0GC can be created without direct use of seed of inbred line PH0GC. Applicant directs the Examiner to the following web site which further explains and illustrates double haploid technology at the internet address [www.uni-hohenheim.de/%7Eipspwww/350b/indexe.html#Project3](http://www.uni-hohenheim.de/%7Eipspwww/350b/indexe.html#Project3) (attached as Appendix 1), as well as to U.S. Patent No. 5,770,788 to Jia and U.S. Patent No. 6,200,808 to Simmonds *et al.*. As noted on the web site, the use of double haploid technology to has been used in plant breeding to produce desired homozygous inbred lines for more than 50 years.

Claim 25 is rejected as indefinite "because it is directed to a maize plant derived from inbred line PH0GC, but is dependent upon claim 11 that is not specifically directed to inbred line PH0GC, only a maize plant having all of the physiological and morphological characteristics of PH0GC".

Applicant traverses this rejection for the reasons asserted *supra*. Claim 25 is definite and does include the plant of claim 11 wherein the plant is "[a] maize plant having all the physiological and morphological characteristics of inbred line PH0GC, wherein a sample of the seed of inbred line PH0GC was deposited under ATCC Accession Number PTA-4523". In addition, claim 25 claims the maize plant of claim 11 with these additional limitations, which are not necessarily present in the maize plant of claim 11. The presence of these additional limitations does not mean that claim 25 does not possess all limitations of claim 11; these claims still require a maize plant having the physiological and morphological characteristics of inbred line PH0GC. Because claim 25 does incorporate all elements of claim 11, it is in accordance with the requirements of § 112, second paragraph.

Claims 28 and 29 are rejected as indefinite "because it is unclear what the metes and bounds of employing the maize plant of claim 11 are".

Applicant traverses this rejection for the reasons asserted *supra*. Claims 28 and 29 are definite and do include the plant of claim 11. Thus, because claims 28 and 29 do incorporate all elements of claim 11, it is in accordance with the requirements of § 112, second paragraph.

Claim 30 is rejected as the Examiner states it is indefinite "because the method requires 'obtaining an F1 hybrid seed from which maize inbred line PH0GC is a parent', but said claim is dependent upon claim 11 which is directed to a 'maize plant having all the physiological and morphological characteristics of inbred line PH0GC'".

Applicant traverses this rejection for the reasons asserted *supra*. Claim 30 is definite and does incorporate all elements of claim 11, and therefore it is in accordance with the requirements of § 112, second paragraph.

In light of the above amendments and remarks, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, second paragraph.

#### **Rejections Under 35 U.S.C. § 112, First Paragraph**

##### ***A. Written description regarding Claims 1-10 and 11-30***

Claims 1-10 remain rejected and claims 11-30 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter, which was not described in the specification in such a way as reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The Examiner states the rejection is repeated for the reasons of record set forth in the Office Action of July 21, 2005. *See* Office Action, pp. 4-8.

Applicant respectfully traverses this rejection. Applicant reiterates that the written description requirement of § 112, first paragraph has been fulfilled by depositing seeds of PH0GC in a public depository and by referencing the deposit in the specification. *See* specification, p. 76, ll. 2-28; *see also Enzo Biochem, Inc. v. Gen-Probe Inc.*, 323 F.3d 956, 965, 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002) (stating that the written description requirement of § 112, ¶ 1 may be fulfilled by depositing material in a public depository, where the deposited material is not accessible in writing, and where reference to the deposit is made in the specification). This deposit not only describes inbred maize line PH0GC but also the hybrid maize plants, plant parts, and seeds grown in claims 1-10 and 11-30. In a prior case before the Board of Patent Appeals and Interferences, the Board determined that where claims to an inbred

maize plant satisfied the written description requirement, claims to the F1 hybrid seed and plants with the inbred maize plant as a parent also satisfied the written description requirement. *See Ex parte Carlson* (B.P.A.I. 2005). The Board therein stated:

All that is required by the claims is that the hybrid has one parent that is a plant of corn variety [inbred]. Since the examiner has indicated that the seed and the plant of the corn variety [inbred] are allowable . . . there can be no doubt that the specification provides and adequate written description of this corn variety. In addition, the examiner appears to recognize (Answer, page 25) that appellant's specification describes an exemplary hybrid wherein one parent was a plant of the corn variety [inbred]. . . Accordingly, it is unclear to this merits panel what additional description is necessary.

*Ex parte Carlson*, p. 16. Here, Applicant has done just what the applicant in *Ex parte Carlson* did, that is claim hybrids having one parent that is a plant of an inbred variety. Further, Applicant reiterates that the specification contains an example of a hybrid produced by PH0GC in the application as filed. *See* specification, p. 38, Table 3. Thus, under *Ex parte Carlson*, "it is unclear . . . what additional description is necessary." *See Ex parte Carlson*, p. 16; *see also Regents of Univ. of Cal.*, 119 F.3d at 1569, 43 U.S.P.Q.2d at 1406 (stating that an Applicant is "not required to disclose every species encompassed by their claims even in an unpredictable art").

Applicant reiterates that each member of the genus of hybrids which has PH0GC has a parent and which is encompassed by claims 1-10 and 11-30 shares the identifying structural feature of the cells and/or chromosomes of inbred line PH0GC. An Applicant's claims are described where they set forth and define "structural features commonly possessed by members of the genus that distinguish them from others." *Regents of Univ. of Cal. v. Eli Lilly & Co.*, 119 F.3d 1559, 1568, 43 U.S.P.Q.2d 1398, 1406 (Fed. Cir. 1997) (emphasis added). One of skill in the art, utilizing technology well known in the art, could identify any member of the claimed genus.

The Examiner again cites *In re Wallach*, 71 USPQ2d 1939 at 1940 (C.A.F.C. 2004).

Applicant respectfully traverses and reiterates that the *Wallach* case is not applicable to the claimed invention. Unlike in *Wallach*, the issue in the present case is the characterization of an entire genome, not a single isolated protein. Those of skill in the art utilize molecular markers, such as SSR's, to characterize plant genomes. As Applicant clearly teaches in the specification:

"In addition to phenotypic observations, a plant can also be identified by its genotype. The genotype of a plant can be characterized through a genetic marker profile, which can identify plants of the same variety or a related variety or be used to determine or validate a pedigree. Genetic marker profiles can be obtained by techniques such as... Simple Sequence Repeats (SSRs)...For example, see Berry, Don, et al., "Assessing Probability of Ancestry Using Simple Sequence Repeat Profiles: Applications to Maize Hybrids and Inbreds", *Genetics*, 2002, 161:813-824, which is incorporated by reference herein." *See* specification, p. 16, lines 23.

The use of molecular marker profiles by those of ordinary skill in the art in backcrossing is also clearly supported by the scientific literature. For example, see Ragot, M. *et al.* (1995) Marker-assisted backcrossing: a practical example, in *Techniques et Utilisations des Marqueurs Moleculaires (Les Colloques, Vol. 72*, pp. 45-56 (attached as Appendix 2), and Openshaw *et al.*, (1994) Marker-assisted Selection in Backcross Breeding, Analysis of Molecular Marker Data, pp. 41-43 (attached as Appendix 3). Specifically, Ragot *et al.* concludes that "recovery of the recurrent parent genotype could proceed even faster than in the experiment described herein, should the appropriate protocol and resources (population size, number and position of markers) be allocated." Therefore, one of ordinary skill in the art can obtain the unique SSR profile of PH0GC which can be used to identify essentially derived varieties and other progeny lines developed from the use of PH0GC, as well as cells and other plant parts thereof.

The Examiner further states that the instant disclosure "only provides an adequate written description for inbred maize line PH0GC, because the functional characteristics of an F1 progeny would be correlated as much with the second parent as with inbred maize line PH0GC". *See* Office Action, pp. 6-7.

Applicant respectfully traverses this rejection. Applicant reiterates that each F1 hybrid which has PH0GC as a parent and which is encompassed by claims 1-10 and 11-30 contain at least one set of chromosomes of inbred line PH0GC. Thus, these claims set forth "structural features commonly possessed by members of the genus that distinguish them from others," as only F1 hybrids with PH0GC as a parent would have a complete set of PH0GC chromosomes. *Regents of Univ. of Cal.*, 119 F.3d at 1568, 43 U.S.P.Q.2d at 1406. The claimed F1 hybrids are therefore described in such a way that distinguishes them from other hybrids, which is sufficient to meet the written description requirement. *See id.*

Further, at its foundation, the written description requirement serves an evidentiary function of making certain that the Applicant is in possession of a specific characteristic that identifies their claimed invention. The data provided by Applicant in Tables 1 and 2-4 serves this purpose. See specification, pp. 18-20 Table 1; pp. 37-39 Tables 2-4. The other inbred is not the point of patentability, nor is it what is being claimed. Rather, the relevant claims are drawn precisely to what is described, inbred maize line PH0GC including the hybrid maize plants, plant parts, and seeds grown in claims 1-10 and 11-30.

It is undisputed that fingerprinting with molecular markers is widely used for characterizing germplasm. Specifically, SSR profiles are known and can be practiced by one of ordinary skill in the art in maize breeding. One of ordinary skill has been enabled by the deposit to make and use minor variants of inbred maize line PH0GC, and one of ordinary skill in the art uses SSR markers to characterize backcross conversions of an inbred. Applicant has claimed in the manner used by those of ordinary skill in the art to characterize backcross conversions. Thus, Applicant respectfully submits the claimed invention is in accordance with the written description guidelines.

One skilled in the art would thus recognize that Applicant was in possession of the invention described in claims 1-10 and 11-30 as of the filing date of the application. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejections under 35 U.S.C. § 112, first paragraph.

*B. Enablement regarding Claims 1-10 and claims 11-30*

Claims 1-10 remain rejected and claims 11-30 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. The Examiner asserts that the claims(s) contains subject matter, which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The Examiner states the "while being enabling for inbred maize line PH0GC, deposited under ATCC Accession No. PTA-4523 and methods of using, does not reasonably provide enablement for a seed comprising at least one set of chromosomes of maize inbred line PH0GC as broadly claimed." The Examiner states the rejection is repeated for the reasons of record set forth in the Office Action of July 21, 2005. See Office Action, pp. 8-11.

Applicant respectfully traverses. Applicant maintains the arguments submitted in the previous Amendment of May 17, 2005 regarding the references (Kevern, Carlone, Segebart '719 and Segebart '109) mentioned by the Examiner.

The Applicant further asserts the specification provides a description of how to backcross traits into PH0GC (Specification, p. 22, l. 33 through p. 23, l. 17) and it is understood by those of skill in the art that backcross conversions are routinely produced and do not represent a substantial change to a variety. The World Seed Organization, on its web site, writes, "[t]he concept of an essentially derived variety was introduced into the 1991 Act of the UPOV Convention in order to avoid plagiarism through mutation, multiple back-crossing and to fill the gap between Plant Breeder's Rights and patents." ASSINSEL, an International breeders association, has published a position paper that refers to a conversion produced by repeated backcrossing of parental lines of hybrid varieties as a "cosmetic modification". As determined by the UPOV Convention, "essentially derived varieties may be obtained for example by the selection of a natural or induced mutant, or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, backcrossing, or transformation by genetic engineering" (emphasis added). Copies of web pages with these quotes are provided in Appendix 4. Thus, it is clear that there is worldwide agreement that by obtaining the seed of a newly developed variety such as PH0GC, and by using such seed for repeated backcrossing in accordance with the current claims, one is producing only a cosmetic modification and plagiarizing the work of the inbred inventor.

The ability of one of ordinary skill in the art to effectively use backcrossing to introgress a single locus conversion is also clearly supported by the scientific literature. For example, see Ragot, M. *et al.* (1995) Marker-assisted backcrossing: a practical example, in *Techniques et Utilisations des Marqueurs Moleculaires (Les Colloques*, Vol. 72, pp. 45-56 (attached as Appendix 2), and Openshaw *et al.*, (1994) Marker-assisted Selection in Backcross Breeding, Analysis of Molecular Marker Data, pp. 41-43 (attached as Appendix 3). Specifically, Ragot *et al.*, demonstrates that "spectacular" progress toward the recurrent parent genotype was obtained with 61 RFLP markers. Ragot *et al.* concludes that "recovery of the recurrent parent genotype could proceed even faster than in the experiment described herein, should the appropriate protocol and resources (population size, number and position of markers) be allocated."

Furthermore, the specification teaches multiple ways of introgressing or transforming a maize plant with various genes which encode specific protein products which confer advantageous traits desired in the plant. (See generally, specification, p. 22-33).

Accordingly, Applicant submits that claims 1-10 and 11-30 are fully enabled and have fully satisfied the legal standards for enablement. Applicant respectfully requests reconsideration and withdrawal of the enablement rejections under 35 U.S.C. § 112, first paragraph.

### **Conclusion**

In conclusion, Applicant submits in light of the above amendments and remarks, the claims as amended are in a condition for allowance, and reconsideration is respectfully requested. If it is felt that it would aid in prosecution, the Examiner is invited to contact the undersigned at the number indicated to discuss any outstanding issues.

Please charge Deposit Account No. 26-0084 for the amount of \$120.00 for a one month extension of time from April 12, 2006 to May 12, 2006, under the provision of 37 C.F.R. § 1.136(a). No other fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,



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Attorneys of Record

**Application of the *in-vivo*-haploid induction in hybrid maize breeding**

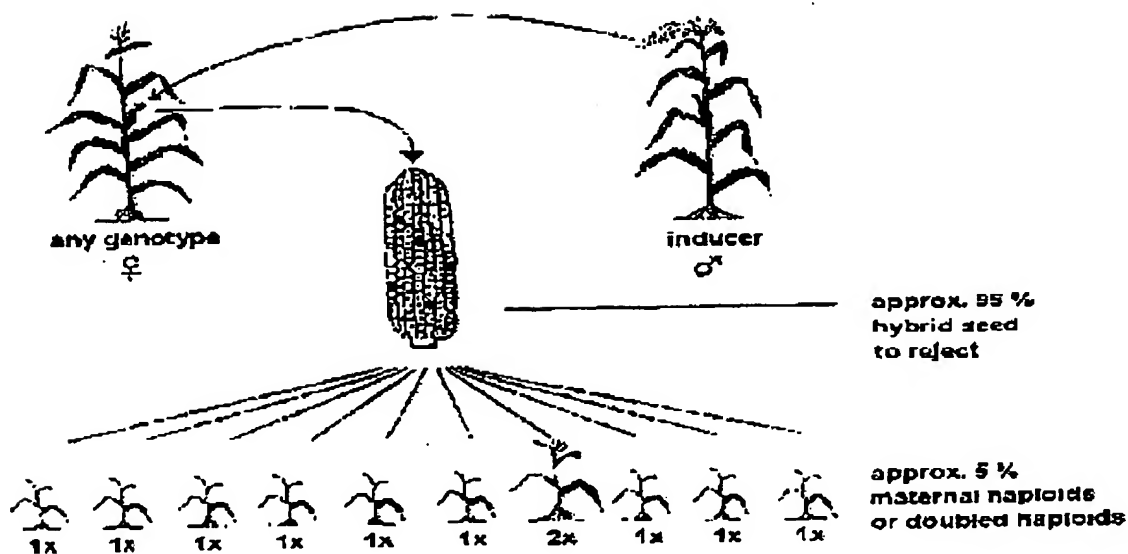
# 1. Reproductive and genetic investigations on *in-vivo*-haploid induction in maize (*Zea mays* L.)

△ Contact person:  
Prof. Dr. H.H. Geiger ([geigerhh@uni-hohenheim.de](mailto:geigerhh@uni-hohenheim.de))



DH-Line In generation D<sub>1</sub>

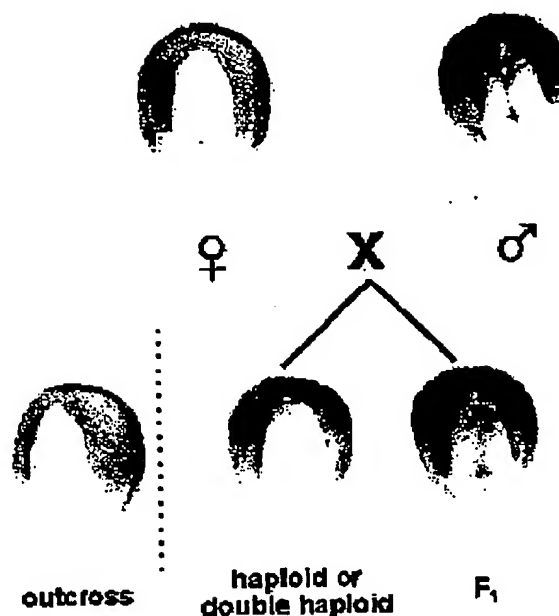
The interest in haploid/double haploid (H/D) techniques has enormously increased in the last years. The introduction of H/DH-techniques in maize breeding programs traces back to the 50s. Shortly after the first reports of the spontaneous occurrence of H/DH-plants in maize, scientists and breeders started to discuss the application of such homozygous plants in breeding programs and their commercial use. By means of the development of inducers and a method for artificial doubling of the chromosome set, the H/DH-technique has been developed in the past years until such an extent that it is being used as a matter of routine by maize breeders.



After pollination with an inducer plant, kernels with H-embryo of maternal origin with triploid endosperm arise, together with regularly

## Present Research Projects in the Department of Population Genetics

double fertilized kernels. Chromosome elimination and parthenogenesis are considered to be the possible biological mechanisms responsible for the occurrence of H-plants. However, chromosome elimination and parthenogenesis exclude each other per definition. Therefore, we chose the neutral term *in-vivo-haploid* induction for the phenomenon mentioned.



Inductor RWS

The aim of our work was to develop a novel inducer line with an increased induction rate. The inducer line RWS developed, displays both advantage of a high induction rate and combination of two dominant identification markers: a red stem, and an embryo and endosperm coloration. Inducer RWS enables the breeder to use *in-vivo-haploid* induction as an effective tool for development of H/DH-plants with almost any genetic background. The method is less effective with donor genotypes, carrying the above mentioned identification markers or anthozya inhibitor-genes themselves.

The spontaneous doubling rate in maize ranges from 1-10 %. Therefore an artificial chromosome doubling method to increase the number of fertile DH-plants is essential. The artificial chromosome doubling method, using colchicine as doubling agent, facilitates an effective development of DH lines.

Present Research Projects in the Department of Population Genetics

Page 5 of 20



Identification of H/DH-plants based on lacking stem-coloration



H/DH-field

in *Arabidopsis*. In *Methods in Arabidopsis* 2.

IM, GOODMAN H.M., KOORNNEEF  
MYEROWITZ R.M., 1993. An integrated  
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CANOCHIE M., MOISAN A., JOURJON  
JER D., GIRAUDAT J., GUIGLEY P.,  
POIX E., GUILLET F., DELSANT M.,  
BLICK J., PHILIPPE G., AXELLOS M.,  
An inventory of 1152 expressed sequences  
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SCHMIDT R., CHOPS G., DEAN C.,  
ANTOFF L., SOMERVILLE C., 1991.  
Index of the *Arabidopsis* genome. *Plant J.*,

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LOS W.D.B., HANCOCK R.M., GOODMAN  
Mapping of *Arabidopsis thaliana*. *Plant Cell*,

2, 9, 111-127.

Isolation of an overlapping YAC library of  
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Techniques of utilisation des marqueurs moléculaires  
Marpeller (France), 28-31 mars 1994  
Ed. INRA, Paris 1666 (Les Colloques, n°72)

## Marker-assisted backcrossing: a practical example

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### Summary

That molecular markers allow fast recovery of recurrent parent genotype in backcross programs is undisputed. Restriction Fragment Length Polymorphism (RFLP's) were used to introgress by backcross a transgene construct, containing phosphinothricin resistance and insecticidal protein genes, from a transformed parent into an elite inbred line. At each generation plants carrying the transgene construct were selected based on their phosphinothricin resistance, and further characterized with RFLP's. Both maximum recovery of recurrent parent genotype and minimum linkage drag were taken into account for marker-based selection. Embryo rescue was used to shorten generation time. Progress towards recurrent parent genotype was spectacular. Levels of recurrent parent genotype recovery which would normally be observed, in the absence of selection, in the BC<sub>2</sub> generation were obtained at the BC<sub>2</sub> generation, about one year after BC<sub>1</sub> seeds had been planted. Besides the evidence already provided by RFLP's, phenotypic evaluation of the backcross-derived near-isogenic lines will constitute an additional check of the completeness of the conversion.

### Introduction

Backcrossing has been a common breeding practice for as long as elite germplasm has been available. It has mainly been used to introgress single Mendelian traits, such as disease resistance or quality factors, into elite germplasm (Allard 1960; Hallauer and Miranda 1981). One of the most attractive attributes of backcrossing is that it allows to perform targeted modifications without disrupting the existing overall genetic balance of the recurrent parent.

However, production of fully converted near isogenic lines through classical backcrossing procedures is a lengthy procedure, if at all possible. Theoretically, a minimum

of seven classical backcross generations are required to recover more than 99% of recurrent parent genotype, assuming no linkage drag. The attractiveness of classical backcross procedures is therefore substantially diminished for crops, such as maize (*Zea mays* L.), where the turn-over of elite cultivars is very fast. In addition, full recovery of recurrent parent genotype is usually not achieved through classical backcrossing, which may result in deleterious agronomic effects. Murray *et al.* (1988) reported about 90% recurrent parent genotype recovery in two BC<sub>3</sub>-equivalent conversions (A632Ht and A632Rp) of the maize line A632. The conversions had retained respectively 4 and 7 donor fragments in addition to the one carrying the gene of interest.

Reduction in the number of backcross generations needed to obtain fully converted individuals has been shown theoretically, or from simulations, to be achievable through the use of molecular markers (Tankley *et al.* 1989; Hospital *et al.* 1992; Jarosz *et al.* 1994). Because they provide thorough characterization of the genetic variability at each backcross generation, markers allow to take full advantage of this variability by applying the highest possible selection intensity.

Efficiency of marker-assisted backcrossing was investigated through an experiment aimed at introgressing a single genetic factor (a transgene construct) from a donor into a recipient maize line.

## Materials and methods

### Plant Material

A hemizygous transgenic maize line of Lancaster origin was used as donor parent to introgress its transgene construct, through repeated backcrossing, into a recipient parent from the Stiff Stalk germplasm group. Both parents are proprietary elite lines. The transgenic construct carries both a phosphinothricin resistance gene and synthetic genes encoding the entomotoxic fragment of the CryIA(A) *Bacillus thuringiensis* protein (Kozel *et al.* 1993). Transformation was achieved through microprojectile bombardment (Kozel *et al.* 1993) and resulted in a single insertion (*Bt locus*), on chromosome 1 (Figure 1).

### Backcross protocol

The F1 progeny of the cross between the donor and the recipient was screened for the presence of the transgene construct by applying Basta, a phosphinothricin-based herbicide, onto each plant. Resistant individuals were then used to generate BC<sub>1</sub> progeny.

For each backcross generation, except the BC<sub>4</sub>, individuals were planted in multipots and sprayed with Basta to eliminate those which did not carry the transgene construct. To avoid the stress resulting from treatment with Basta, BC<sub>4</sub> plants carrying the transgene construct were identified using Southern blots probed with the *pat* and *Bt* genes. Resistant plants were transplanted in an open-soil greenhouse and leaf-sampled for molecular marker

analyses. Results of marker analysis were used to select plants for flowering. A single plant was rescued and transferred into the culture medium, before being averaged, four months.

### Molecular marker analysis

Restriction Fragment Length Polymorphism (RFLP) analysis was performed on all four genotypes in all four genotypes. I were chosen from among those : provided coverage of the entire genome contained two loci tightly linked recombination units away (Fig. BC<sub>n+1</sub> generation comprised by or tightly linked ones, and add selected BC<sub>n</sub> plant was heterozygous independent reference population generation.

### Selection procedure

At each generation plants recurrent-parent-genotype and attempt to integrate both criteria missing values were not include contributed to the selection procedure best ranking one of those for n for the BC<sub>3</sub> selection) was available

## Results and discussion

### Selection for the gene or

The observed segregation significantly different ( $P < 0.05$ )

### Recurrent parent genotype

Statistics for the genotype performed using the whole BC backcross-derived plant there

recover more than 99% of recurrent  
effectiveness of classical backcross  
ops, such as maize (*Zea mays* L.),  
in addition, full recovery of recurrent  
backcrossing, which may result in  
recovered about 90% recurrent parent  
(A632Ht and A632Rp) of the maize  
and 7 donor fragments in addition to

as needed to obtain fully converted  
lines, to be achievable through the  
et al. 1992; Jarosz et al. 1994).  
genetic variability at each backcross  
variability by applying the highest

investigated through an experiment  
the construct) from a donor into a

origin was used as donor parent to  
backcrossing, into a recipient parent  
are proprietary elite lines. The  
donor gene and synthetic genes  
the *Sturtevant* protein (Kozel et  
projectile bombardment (Kozel et  
chromosome 1 (Figure 1).

the recipient was screened for the  
phosphinothricin-based herbicide,  
in BC<sub>1</sub> progeny.

Individuals were planted in multipots  
carry the transgene construct. To  
BC<sub>1</sub> plants carrying the transgene  
the *per* and *Bt* genes. Resistant  
leaf-sampled for molecular marker

analyses. Results of marker analyses were made available at the latest two weeks after  
flowering. A single plant was selected, of which all backcross-derived embryos were  
rescued and transferred onto tissue culture medium. Plantlets that developed from these  
embryos first underwent a greenhouse acclimation phase, while still growing on tissue  
culture medium, before being transplanted into multipots. Backcross cycles lasted, on  
average, four months.

#### Molecular marker analyses

Restriction Fragment Length Polymorphisms (RFLP's) were used to establish  
genotypes in all four generations. RFLP detection involved either radioactive or  
chemiluminescent techniques. For the BC<sub>1</sub> generation, 68 marker-enzyme combinations  
were chosen from among those revealing polymorphism between donor and recipient. They  
provided coverage of the entire genome, defining intervals of about 25 cM in size, and  
contained two loci tightly linked to the *Bt* locus, CG320 and CG415, respectively 5 and 16  
recombination units away (Figure 1). For subsequent generations, markers analyzed in the  
BC<sub>n+1</sub> generation comprised both those for which the selected BC<sub>n</sub> plant was heterozygous,  
or tightly linked ones, and additional ones located in chromosomal segments for which the  
selected BC<sub>n</sub> plant was heterozygous (Table 1). Marker map positions were obtained from  
independent reference populations and confirmed by analysis of segregation in the BC<sub>1</sub>  
generation.

#### Selection procedure

At each generation plants were ranked based both on the percentage of homozygous  
recurrent-parent-genotype and on the extent of linkage drag around the *Bt* locus, in an  
attempt to integrate both criteria. Plants for which two or more adjacent markers had  
missing values were not included in the analysis. Success or failure of the pollinations also  
contributed to the selection procedure. One single plant was selected at each generation: the  
best ranking one of those for which a backcross progeny of size 100 or more (50 or more  
for the BC<sub>1</sub> selection) was available.

#### Results and discussion

##### Selection for the gene of interest

The observed segregation ratios for phosphinothricin resistance (Table 1) were not  
significantly different ( $P < 0.05$ ) from the expected 1:1, as shown by Chi-square tests.

##### Recurrent parent genotype recovery

Statistics for the genotyped plants are summarized in Table 1. Calculations were  
performed taking the whole genome into account, including the *Bt* locus. The "perfect"  
backcross-derived plant therefore carries one heterozygous chromosomal segment, that



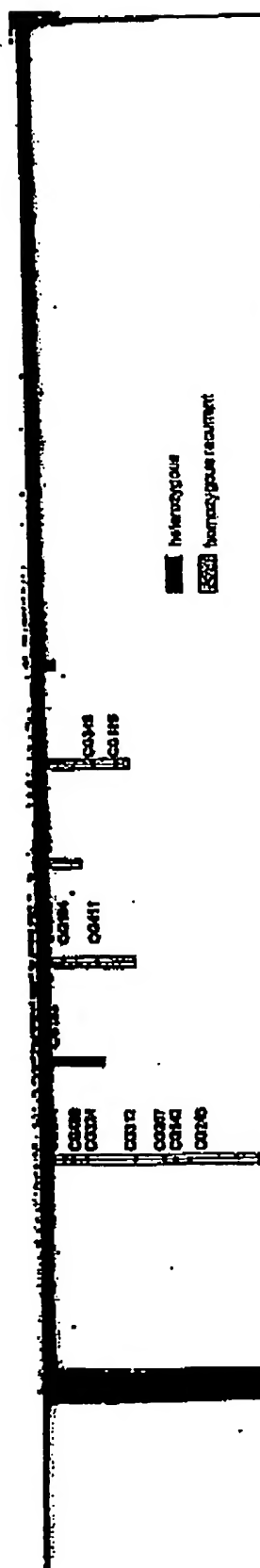


Figure 1a Genetic maps of the backcross-derived individuals selected in the first four generations of a marker-assisted backcross program. The locus to be introgressed (Bt) is located on chromosome 1.

### SELECTED BC2

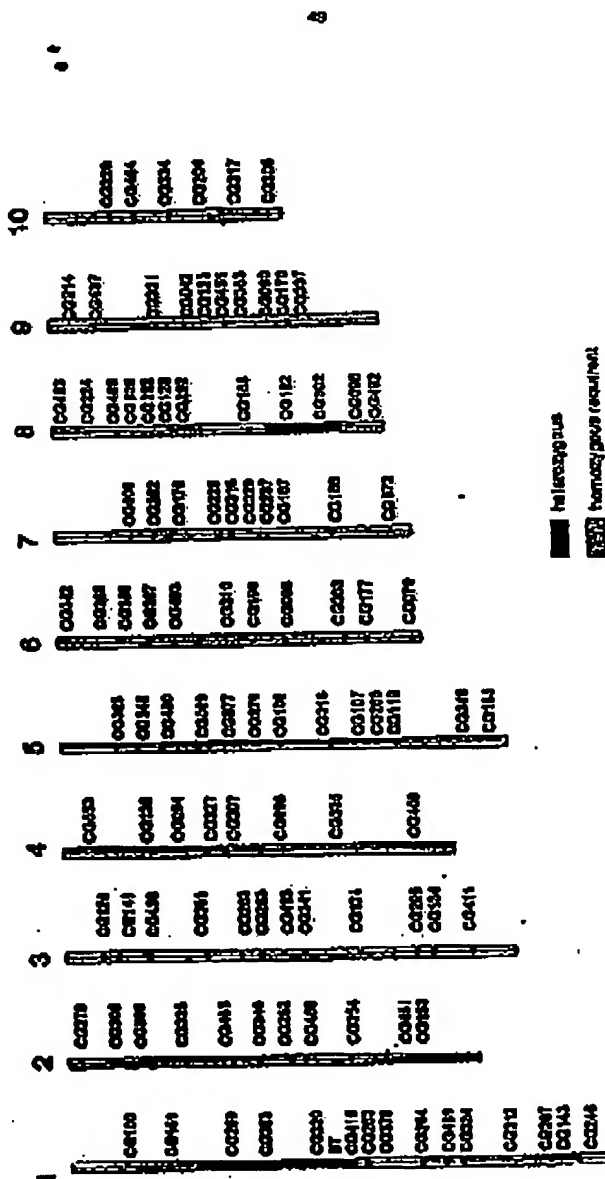
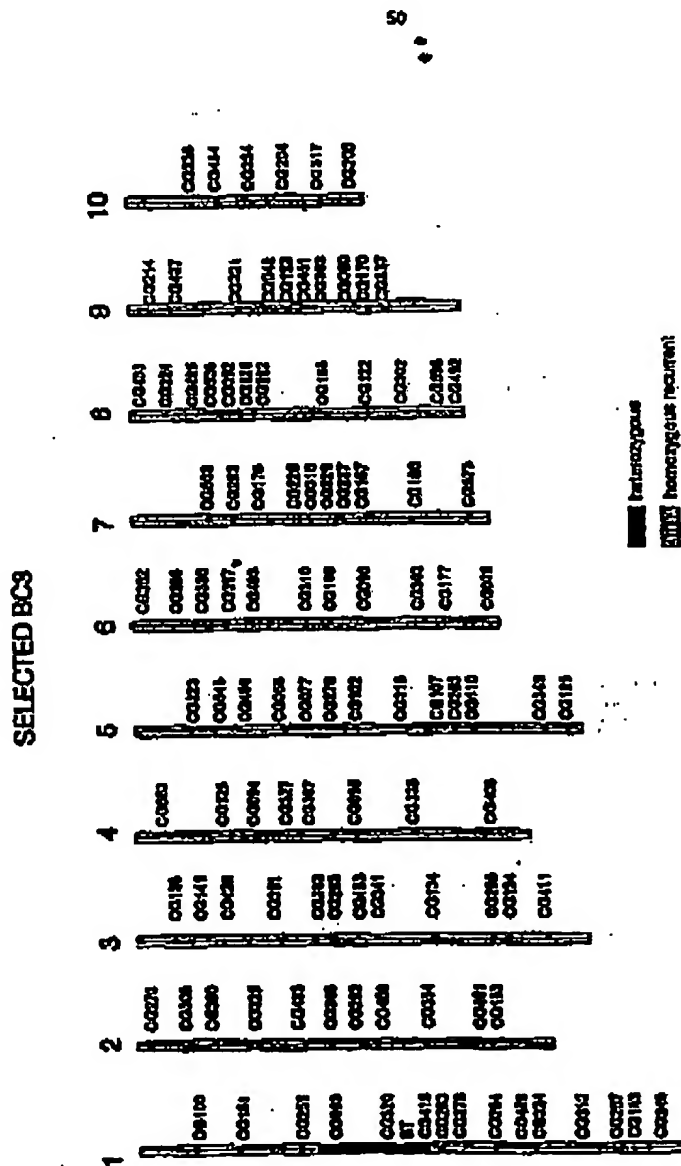
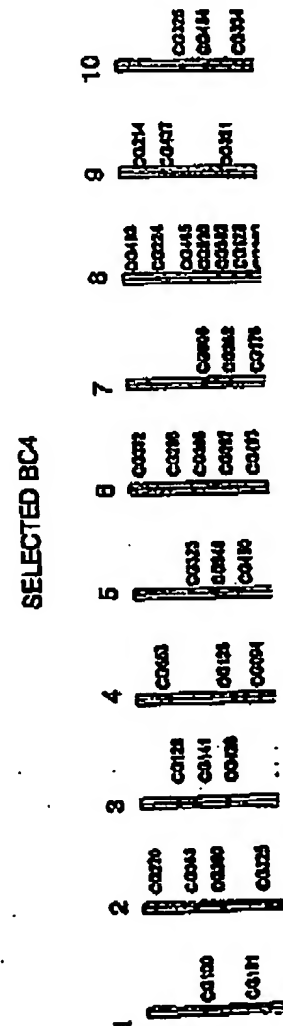


Figure 1b Genetic maps of the backcross-derived individuals selected in the first four generations of a marker-assisted backcross program. The locus to be introgressed (Bt) is located on chromosome 1.



Figures 1-c) Genetic maps of the bacterium-derived individuals selected in the first four generations of a matrix-assisted bacteriophage program. The locus to be investigated (*St*) is located on chromosome I.



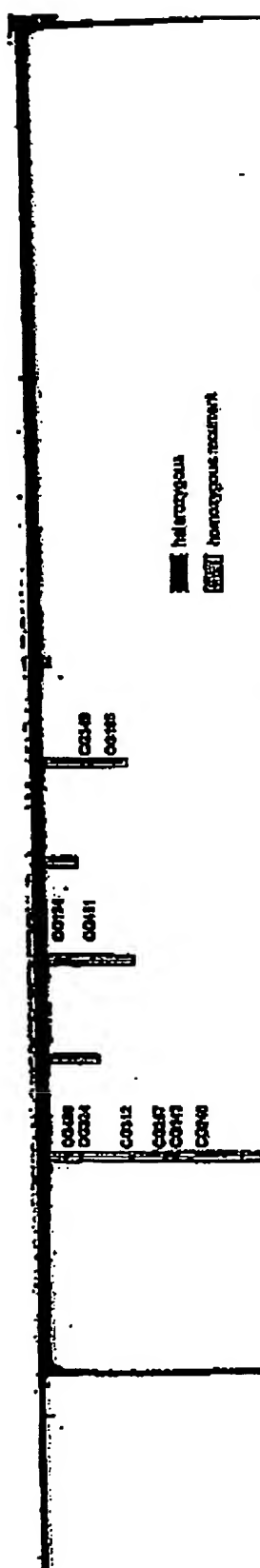


Figure 1-c) Growth curve of the bacterium-derived individuals selected in the first four generations of a counter-selected bacteriophage program. The locus to the bacteriophage ( $\lambda$ ) is located on chromosome I.

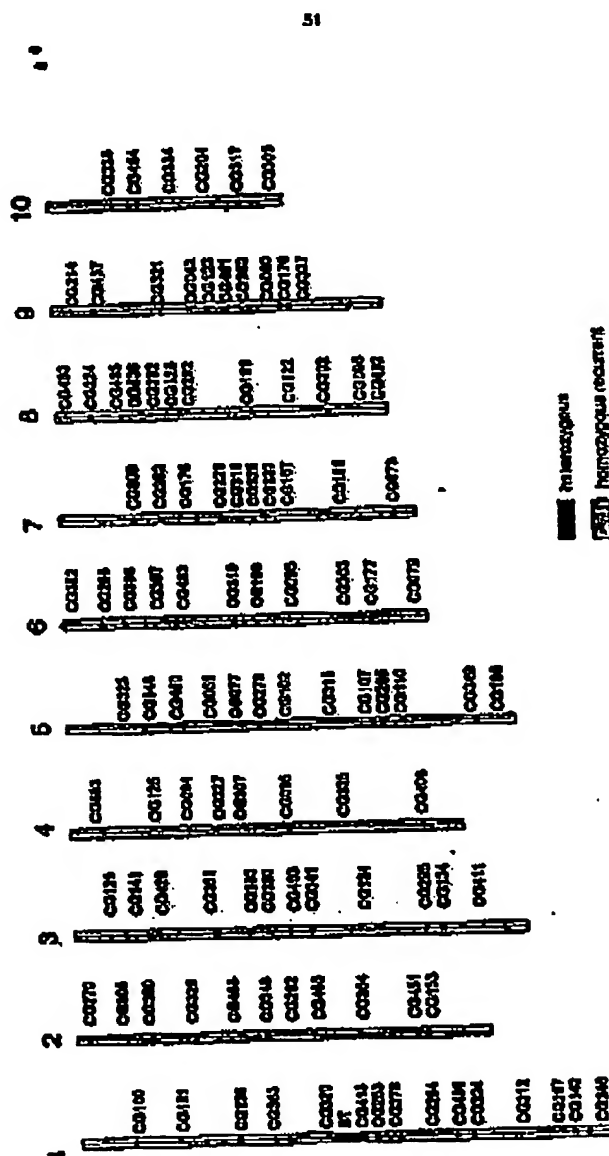


Figure 1-41 Genetic maps of the bacteriophage individuals selected in the first four generations of a marker-selected bacteriophage program. The locus to be transferred (B<sub>2</sub>) is located on chromosome 1.

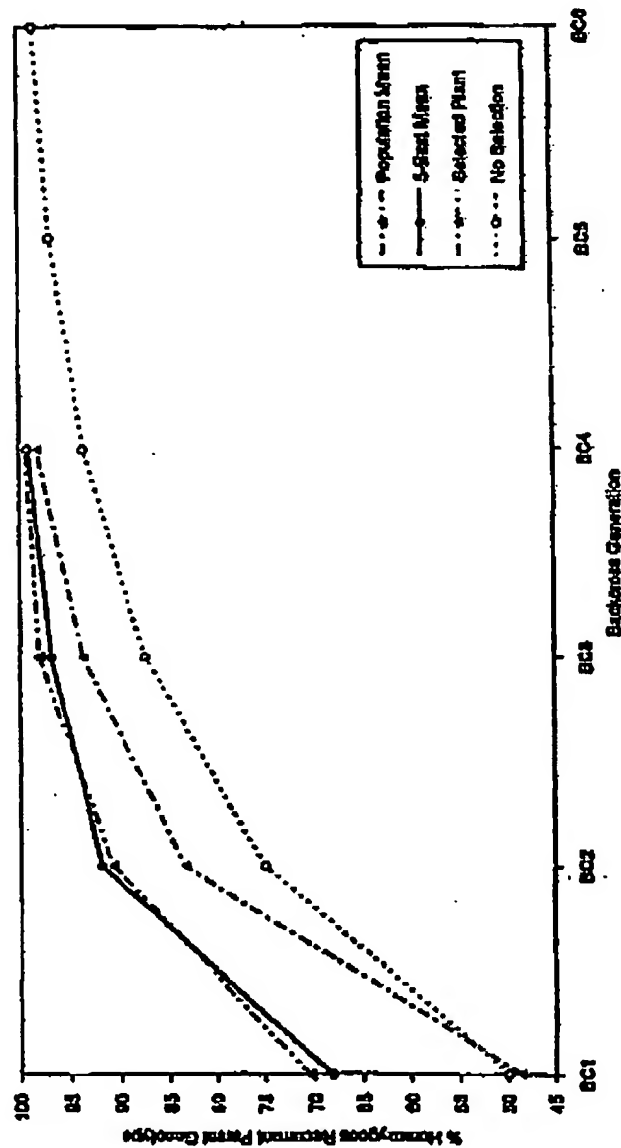


Figure 2: Recovery of recurrent parent genotype through backcrossing, with or without empirically-assisted selection.

Table 1: Proportion and characteristics of plants carrying the genes of interest, in the first four generations of a marker-assisted backcross program.

generation	% homozygous	% heterozygous	% plants	% homozygous recurrent	% heterozygous
...					

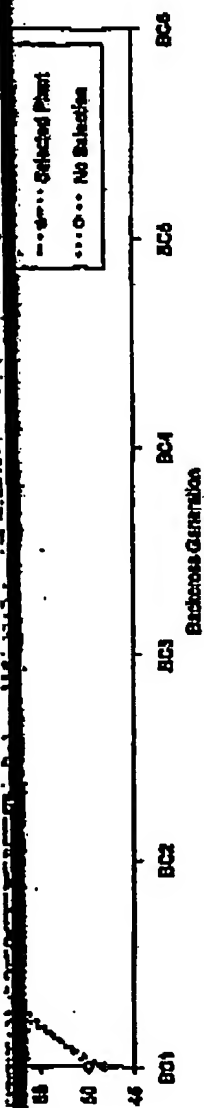


Figure 2: Recovery of recurrent parent genotype through backcrossing, with or without marker-assisted selection.

Table 1: Proportion and characteristics of plants carrying the genes of interest, in the first four generations of a marker-assisted backcross program.

Generation	W phosphatidic acid resistant plants	RFLP genotyping			nb plants analyzed *	W homozygous recurrent parent genotype				nb heterozygous chromosome segments ***			
		nb plants	nb ind	nb despolids		mean	std dev	5-best ind	selected plant	mean	std dev	5-best mean	selected plant
BC1	48.03	96	81	86.66	87	48.72	10.35	88.31	70.45	11.01	2.17	7.73	8
BC2	44.63	81	22	124.2	30	63.42	8.84	81.93	92.44	3.05	1.54	3.30	3
BC3	48.33	72	10	72.0	26	83.83	1.81	86.82	88.93	2.39	0.71	1.85	1
BC4		28	3	78	28	84.28	0.43	85.08	88.38	1.59	0.60	1.00	1

\* Plants for which two or more adjacent markers had missing values were not included in the analyses.

\*\* Mean value of the five individuals having the five highest percentages of homozygous recurrent parent genotype.

\*\*\* Including the segments carrying the transgene construct.

comprising the *Bt* locus. It also displays 99.36% of homozygous recurrent-parent-genotype. The remaining 0.64% corresponds to the average relative length of the chromosome segment containing the *Bt* locus, which depends on the two flanking markers chosen.

The mean percentage of homozygous recurrent-parent-genotype of the  $BC_1$  generation was slightly lower than the expected 50%. This can be explained by linkage drag around the *Bt* locus, given that this percentage was composed based only on plants selected for heterozygosity at the *Bt* locus. For all other backcross generations the mean percentage of homozygous recurrent-parent-genotype was much higher than what would have been observed, should no selection have been done (Figure 2).

The percentage of homozygous recurrent-parent-genotype of the selected plant (Table 1) and the average of the five largest values (Table 1) were always very similar to one another, and much superior to the population mean values (Figure 2). The percentage of homozygous recurrent-parent-genotype of the selected plant was found only once, in the  $BC_2$  generation, to be smaller than the average of the five largest values. This corresponded to the only time when the selected plant was not the one with the maximum percentage of homozygous recurrent-parent-genotype. This plant had been selected because it displayed a favorable recombination on one side of the *Bt* locus (Figure 1).

The percentage of homozygous recurrent-parent-genotype of the selected  $BC_1$  plant was almost equal to that of an unselected  $BC_1$ , that of the selected  $BC_2$  was larger than that of an unselected  $BC_2$ , that of the selected  $BC_3$  was barely smaller than that of an unselected  $BC_3$ , and that of the selected  $BC_4$  was equal to that of the "perfect" backcross-derived plant, given the set of markers that was used. Such rates of recurrent parent genotype recovery are consistent with results of simulation analysis. Jarboe *et al.* (1994) also used the maize genome as a model reported that three backcross generations and 80 markers were needed to recover 99% of recurrent parent genotype.

#### Number of donor chromosome segments

The number of heterozygous chromosomal segments decreased from one backcross generation to the next. Plants selected at each generation were not necessarily those which had the lowest number of heterozygous chromosomal segments (Table 1). However, with the set of markers used,  $BC_3$  and  $BC_4$  plants were recovered which contained only one heterozygous chromosomal segment: that comprising the *Bt* locus.

#### Linkage drag

Linkage drag around the *Bt* locus was estimated, relative to the length of chromosome 1. Its value was found to lie between 24.0 and 48.4% for the selected  $BC_1$  individual, between 17.6 and 34.8% for the selected  $BC_2$ , between 2.0 and 24.0% for the selected  $BC_3$ , and between 0.0 and 8.4% (respectively 0.0 and 14.5 cM) for the selected  $BC_4$ .

The two values given for each  $BC_i$  correspond to extreme positions or flanking the transgene construct locus  $BC_i$  is likely to be less than 1.5% appear to be somewhat high, reflect drag, it is much lower than what (Stam and Zeven 1981; Tanksley *et al.* of tomato cultivars obtained by a  $BC_4$  Tanksley (1989) found that the sizes cM.

#### Conclusion

These results clearly demonstrate quality advantages over classical through backcrossing. Only four years and a half from plant genotypically fully converted. New genotype could proceed even faster appropriate protocol and resources allocated.

Comparison of  $BC_4$ -derived 1 markers and agronomic performance order to confirm the completeness of

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homozygous recurrent-parent-genotype. The relative length of the chromosome between the two flanking markers chosen.

The parent-genotype of the  $BC_1$  generation was explained by linkage drag around the  $At$  locus based only on plants selected for two generations the mean percentage of  $At$  was higher than what would have been expected (2).

The parent-genotype of the selected plant (Table 1) were always very similar to the  $At$  value (Figure 2). The percentage of  $At$  plant was found only once, in the five largest values. This corresponded to one with the maximum percentage of  $At$  had been selected because it displayed a  $At$  (Figure 1).

The parent-genotype of the selected  $BC_1$  plant of the selected  $BC_2$  was larger than that of the  $At$  but smaller than that of an unselected  $At$  of the "perfect" backcross-derived  $At$  rates of recurrent parent genotype analysis. Jarosz *et al.* (1994) who used backcross generations and 80 markers  $At$  type.

Percentages decreased from one backcross generation were not necessarily those which segments (Table 1). However, with  $At$  recovered which contained only one  $At$  locus.

relative to the length of chromosome  $At$  4% for the selected  $BC_1$  individual, from 2.0 and 24.0% for the selected  $At$  (14.5 cM) for the selected  $BC_2$ .

The two values given for each generation are extreme values of linkage drag, which correspond to extreme positions of the crossing-over in the marker-defined intervals flanking the transgene construct locus. Therefore the true linkage drag value of the selected  $BC_1$  is likely to be less than 1.3% of the genome. Although this maximum value may appear to be somewhat high, reflecting the limited selection pressure per  $At$  on linkage drag, it is much lower than what would be expected from classical backcross programs (Stam and Zeevalk 1981; Tanksley *et al.* 1989). Practically, in a study of  $Tm-2$  conversions of tomato cultivars obtained by a large number of classical backcross cycles, Young and Tanksley (1989) found that the sizes of the introgressed fragments ranged between 4 and 51 cM.

### Conclusion

These results clearly demonstrate that molecular markers provide important time and quality advantages over classical procedures for the production of near-isogenic lines through backcrossing. Only four backcross generations were necessary to recover, in less than a year and a half from planting of the  $BC_1$ 's, individuals which appeared to be genotypically fully converted. Nevertheless, it is likely that recovery of recurrent parent genotype could proceed even faster than in the experiment described herein, should the appropriate protocol and resources (population size, number and position of markers) be allocated.

Comparison of  $BC_1$ -derived lines with the recurrent parent for both morphological markers and agronomic performance (including hybrid performance) will be performed in order to confirm the completeness of the conversion.

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# Marker-assisted Selection in Backcross Breeding

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**Abstract.** The backcross breeding procedure has been used widely to transfer simply inherited traits into elite genotypes. Genetic markers can increase the effectiveness of backcrossing by 1) increasing the probability of obtaining a suitable conversion, and 2) decreasing the time required to achieve an acceptable recovery. Simulation and field results indicated that, for a genome consisting of ten 200-cM chromosomes, having selection on 40 or 80 markers in 50 BC individuals that carry the allele being transferred can reduce the number of backcross generations needed from about seven to three.

The backcross breeding procedure has been used widely to transfer simply inherited traits into elite genotypes. Usually, the trait being transferred is controlled by a single gene, but highly heritable traits that are more complexly inherited have also been transferred successfully by backcrossing; for example, maturity in maize (Riba and Senter, 1961; Shaver, 1976). Today, backcrossing is being used to transfer genes introduced by such techniques as transformation or mutation into appropriate germplasm.

Several plant breeding textbooks give good descriptions of the backcross procedure (Allard, 1960; Fehr, 1987). A donor parent (DP) carrying a trait of interest is crossed to the recurrent parent (RP), an elite line that is lacking the trait. The F<sub>1</sub> is crossed back to the RP to produce the BC<sub>1</sub> generation. In the BC<sub>1</sub> and subsequent backcross generations, selected individuals carrying the gene being transferred are backcrossed to the RP. The expected proportion of DP genome is reduced by half with each generation of backcrossing. Ignoring effects of linkage to the selected DP allele being transferred, the percentage recurrent parent (%RP) genome expected in each backcross generation is calculated as:

$$\%RP = 100 [1 - (0.5)^n]$$

where  $n$  is the number of backcrosses.

Backcrossing of selected plants to the RP can be repeated each cycle until a line is obtained that is essentially a version of the RP that includes the integrated allele. After six backcrosses, the expected recovery is >99% (Table 1).

Until recently, discussions of the recovery of the RP genome during backcrossing have emphasized the expected values for

%RP shown in Table 1, and have largely ignored the genetic variation for %RP that exists around the expected mean. With the development of genetic markers capable of providing good genome coverage, there has been interest in taking advantage of that variation to increase the efficiency of backcrossing.

Selection for RP marker alleles can increase greatly the effectiveness of backcross programs by allowing the breeder to 1) select backcross plants that have a higher proportion of RP genome, and 2) select backcross individuals that are better conversions near a mapped donor allele being transferred (i.e., select for less linkage drag). Expressed in practical terms, using genetic markers to assist backcrossing can 1) increase the probability of obtaining a suitable conversion, and 2) decrease the time required to achieve an acceptable recovery.

Issues to consider when planning a marker-assisted backcross program include 1) the time advantage of using markers to assist backcrossing, 2) the number of markers needed, and 3) the number of genotypes to evaluate. In this report, we use results from previous literature, computer simulation, and empirical studies to provide some guidelines.

Table 1. Expected recovery of recurrent parent (RP) genome during backcrossing, assuming no linkage to the gene being transferred.

Generations	%RP
F <sub>1</sub>	50.0000
BC <sub>1</sub>	75.0000
BC <sub>2</sub>	87.5000
BC <sub>3</sub>	93.7500
BC <sub>4</sub>	96.8750
BC <sub>5</sub>	98.4375
BC <sub>6</sub>	99.2188
BC <sub>7</sub>	99.6094

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## Analysis of Molecular Marker Data

## Materials and methods

The maize genome was the model for the simulation. The simulated genome contained ten 200-cM chromosomes. Simulation of crossing over was based on a Poisson distribution with a mean of 2.0 ( $\lambda = 2$ ) (Haggen, 1959), which, on average, generated one cross over for every 100-cM length. The simulations reported here assume no interference. Codominant genetic markers were evenly distributed in the genome and sites of the donor gene were randomly assigned to genome locations. Simulations were conducted with the following parameters:

Number of progeny: 100 or 500.

Backcross generations:  $BC_1$ ,  $BC_2$ , and  $BC_3$ .

Number of markers: 20, 40, 80, or 100.

Number selected to form the next BC generation: 1 or 5.

Selection was based on 1) presence of the donor allele and 2) high %RP. %RP was calculated as the average of the (one or five) selected individuals. Values presented are the mean of 50 simulations.

## Results

In the computer simulation study, all methods modeled greatly increased the speed of recovering the RP genome compared to the expected recovery with no marker-assisted selection (compare Tables 1 and 2). At least 80 markers were required to recover 99% of the RP genome in just three BC generations (Table 2). Use of at least 80 markers and 500 progeny allowed recovery of 98% RP in just two BC generations. Response to selection was diminished only slightly by spreading the effort over five selections. Using markers, the number of backcross generations needed to convert an inbred is

reduced from about seven to three.

By the  $BC_3$  generation, there appears to be no practical advantage to using 500 vs. 100 individuals. If the presence of the donor trait in the backcross individuals can be ascertained before markers are genotyped, then only half the number of individuals indicated in the tables will need to be analyzed.

When a small number of markers are used, they quickly become non-informative; i.e., selection causes the marker loci to become fixed for the RP type before the rest of the genome is fully converted (Table 3; Hospital et al., 1992). This situation was most prominent in the larger populations, where a higher selection intensity placed more selection pressure upon the marker loci. Accordingly, it is of interest to consider how closely the estimation of %RP based on markers reflects the actual genome composition. The combination of estimation of %RP based on fewer markers and subsequent selection tends to bias the estimates upward (compare Tables 2 and 3).

The results from the simulation compare well with real field data. In a typical example, 50  $BC_3$  plants carrying the gene being transferred were genotyped at 83 polymorphic RFLP loci (note that this corresponds to a population size of 100 unselected plants in Tables 2 and 3). The five best  $BC_3$  recoveries had estimated %RP values of 85.9%, 82.7%, 82.0%, 81.4%, and 81.2%. After evaluating 10  $BC_3$  plants from each selected  $BC_3$ , the best  $BC_3$  recovery had an estimated %RP of 94.6%.

## Discussion

The simulations (Table 2; Hospital et al., 1992) and our experience indicate that four markers per 200-cM chromosome is adequate to greatly increase the effectiveness of selection in the  $BC_3$ . However, using only four markers per 200 cM will likely make it very difficult to map the location of the gene of interest. Adequate summarization of this data is an important

Table 2. Percent recovered parent genome during marker-assisted backcrossing.

Generation	100 Progeny				500 Progeny			
	No. markers				No. markers			
	20	40	80	100	20	40	80	100
<i>One selected</i>								
$BC_1$	84.3	84.5	84.2	88.0	83.9	90.7	90.3	90.5
$BC_2$	93.0	95.2	95.8	97.2	96.5	97.7	98.5	98.6
$BC_3$	97.4	97.6	98.9	99.2	97.7	98.3	99.4	99.5
<i>Five selected</i>								
$BC_1$	82.9	85.1	84.9	84.7	87.7	88.1	88.9	88.9
$BC_2$	93.7	95.0	95.8	95.7	96.5	96.8	97.8	97.9
$BC_3$	97.1	98.3	98.8	98.9	97.3	98.5	99.3	99.3

Table 3. Estimates of percent recovered parent genome, based on marker loci.

Generation	100 Progeny				500 Progeny			
	No. markers				No. markers			
	20	40	80	100	20	40	80	100
<i>One selected</i>								
$BC_1$	98.7	97.8	95.6	97.2	100.0	99.1	98.6	98.0
$BC_2$	100.0	99.8	99.3	99.5	100.0	100.0	99.9	98.2
<i>Five selected</i>								
$BC_1$	96.4	96.5	96.2	95.8	100.0	98.5	98.3	98.2
$BC_2$	99.9	99.8	99.3	99.1	100.0	100.0	99.9	99.8

## Analysis of Molecular Marker Data

part of a marker-assisted backcross program. Ideally, the marker used can supply data that can be represented as alleles of loci with known map positions. Estimation of %RP, mapping the position of the locus of interest, and graphical display of the results (Young and Tanksley, 1989) are all useful in understanding and controlling the specific backcross experiment being conducted.

It appears that, with the use of genomic markers, the portion of the RP genome that is not linked to the allele being transferred can be recovered quickly and with confidence. The recovery of RP will be slower on the chromosome carrying the gene of interest. A considerable amount of linkage drag is expected to accompany selection for the RP allele in a backcross program. For a locus located in the middle of a 200-cM chromosome, the length of the RP chromosome segment accompanying selection is expected to be 126, 63, and 28 cM in the BC<sub>1</sub>, BC<sub>2</sub>, and BC<sub>3</sub> generations, respectively (Hanson, 1959; Naveira and Barbudilla, 1992). Our observations support the recommendation of Hospital et al. (1992) that preference be given to the selection for recombinants proximal to the allele of interest, but that selection for recovery of the RP elsewhere in the genome also be considered. This two-stage selection can probably be done quite effectively ad hoc by the breeder once the data is adequately summarized; however, Hospital et al.

suggest ways to incorporate the two criteria into a selection index such that each component of selection is assured appropriate weighting.

Use of genetic markers can greatly increase the effectiveness of backcrossing, and they should be used in any serious backcrossing program if resources are available to the breeder.

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ASSTYNAL



## Essential Derivation and Dependence

### Practical Information

#### WHY THE CONCEPT OF ESSENTIAL DERIVATION?

The 1978 Act of the UPOV Convention (International Union for the Protection of New Varieties of Plants) states that "the authorization by the breeder shall not be required either for the utilization of the [his protected] variety as an initial source of variation for the purpose of creating other varieties or for the marketing of such varieties".

That principle, known as the "breeder's exemption", is essential for continued progress from plant breeding.

However, its implementation has progressively led to some abuses, due to the difficulties involved with assessment of distinctness, based on the text of the Convention (1978) which indicates that, for the basis of a title of protection, "the [new] variety must be clearly distinguishable by one or more important characteristics from any other variety whose existence is a matter of common knowledge ...".

Sometimes, "cosmetic modifications" were enough for protecting a new variety. That was particularly true in the case of mutation of ornamental or fruit plants and of "conversion" by repeated backcrossing of parental lines of hybrid varieties.

In order to improve the situation, in the early 1980's, a debate began on how to improve the system, trying to define "minimum distances" per species, but no consensus was reached. The development of genetic engineering, opened new possibilities for "piracy" of varieties and sped up the revision process of the Convention which, in the Act adopted in 1991, has introduced with the full agreement of breeders' associations, the concept of essential derivation. That concept of essential derivation has two aspects:

- a technical one: the question whether or not a plant variety is to be considered as a variety essentially derived from an initial variety;
- a juridical one: dependence, meaning that no protected acts as defined by the 1991 Act of the UPOV Convention (production, marketing ...) related to the essentially derived variety shall be carried out without the authorization of the owner of the protected initial variety.

#### DEFINITION OF AN ESSENTIALLY DERIVED VARIETY

The 1991 Act of the UPOV Convention states that "a variety shall be deemed to be essentially derived from another variety (the initial variety) when:

1. It is predominantly derived from the initial variety, or from a variety that is itself predominantly derived from the initial variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial

## Essential Derivation and Dependence - Practical Information

Page 2 of 5

variety;

- ii. it is clearly distinguishable from the initial variety and
- iii. except for the differences which result from the act of derivation, it conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety.

Essentially derived varieties may be obtained, for example, by selection of natural or induced mutants, by selection of a somaclonal variant, by selection of variant individual plants in the initial variety, by backcrossing or by transformation (genetic engineering).

ASSINSEL interprets the definition given in the Convention as follows:

a) The technical aspects (matter of facts)

For a variety to be considered as essentially derived, it must fulfil three requirements in relation to the initial variety while retaining the expression of the essential characteristics of the initial variety:

- i. clear distinctness in the sense of the UPOV Convention
- ii. conformity to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety
- iii. predominant derivation from an initial variety.

If one of these requirements is not fulfilled, there is no essential derivation.

The methods of breeding that can be regarded as leading to an essentially derived variety (see the above-mentioned explanatory list) may differ from species to species or even within a species. This may result in different thresholds being required to characterize essential derivation. Thus, conformity should be judged on a species-by-species or even within a species basis.

b) The juridical aspect

The principle of dependence only exists in favour of a protected variety. This means that:

- i. the initial variety must be a protected one
- ii. dependence can only exist from one protected variety alone
- iii. an essentially derived variety can be directly derived from the initial variety or from a variety that is itself predominantly derived from the initial variety. It is possible to have a "cascade" of derivation. However, each essentially derived variety shall only be dependent on one, the protected initial variety. A cascade of dependence shall not exist, the principle having been introduced to better protect the breeder of the initial variety and not those having made derivations from his work.

## Essential Derivation and Dependence - Practical Information

Page 3 of 5

## ASSESSMENT OF ESSENTIAL DERIVATION

The assessment of essential derivation needs to take into account the three criteria mentioned above:

- ⇒ clear distinctness in the sense of the UPOV Convention
- ⇒ conformity to the initial variety in the expression of the essential characteristics that result from the genotype or the combination of genotypes of the initial variety
- ⇒ predominant derivation from an initial variety.

The first criterion will be decided upon by the office in charge of granting a right to the breeder of the variety, according to the UPOV rule of distinctness.

The second criterion could be based on reliable phenotypic characteristics and/or on reliable molecular characteristics: either close relationship in general which could lead to a "conformity threshold" parallel to the minimum distance threshold used for distinctness or only small differences in some simply inherited characteristics. If this second criterion is considered as fulfilled, then, we have to assess the third one, which is "predominant derivation from an initial variety".

The third criterion, predominant derivation from an initial variety, implies that the initial variety or products essentially derived therefrom have been used in the breeding process.

In order to prove that use, various criteria or a combination thereof may be used:

- ⇒ combining ability
- ⇒ phenotypic characteristics
- ⇒ molecular characteristics.

These criteria will have to be handled differently from their use for assessment of distinctness. Whatever solution retained, one will probably have to use distance coefficients to define thresholds. Up to now, ASSINSEL has essentially worked on thresholds based on distances measured by molecular markers. Geneticists and statisticians consider that technically it is equally possible to measure distance coefficients using phenotypic markers. However, the process would probably be more difficult due to environmental factors and much more expensive: necessity of several testing locations during several years. However, if breeders prefer to use morphological markers instead of molecular markers, that should be possible.

The interest of using combining ability and the heterosis level will strongly depend on the crop. Thresholds will also be necessary.

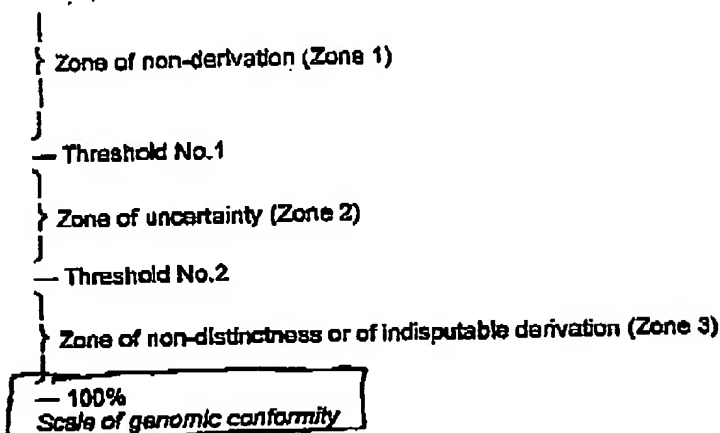
The various ASSINSEL Sections are considering the establishment of thresholds for characterization of essential derivation according to this following general principle:

- ⇒ One should propose, species by species, a first threshold below which a variety should be considered as non-essentially derived from an initial variety and a second threshold of conformity above which the new variety should be considered as essentially derived, except if the breeder can prove, by clear evidence, that he has started from independent germplasm.
- ⇒ Between those two thresholds, the derivation could be disputable and the breeder of the putative essentially derived variety should have to give, in case of amicable negotiation or arbitration, information on the origin of the new variety. Should that information be unsatisfactory, the tribunal or of arbitrators/conciliators agreed on by both parties may request breeding records be provided for their examination.

## Essential Derivation and Dependence - Practical Information

Page 4 of 5

This approach may be diagrammed as follows:



Some breeders are developing such scheme and call the zone No.1 "green zone", in which breeders would have freedom to operate. Zone No.3, the "red zone", where the breeder would know, according to his breeding materials, if his new variety is obviously essentially derived and dependent. Zone No.2 is where there would be uncertainty and where discussion may be appropriate. The threshold levels would be established first as an experiment. They could be further modified according to the experience acquired in the implementation of the scheme.

While this approach may be worthwhile, it also presents some obvious difficulties:

- Breeders have so far been unable to agree on threshold levels for any species;
- Even if the thresholds adopted by the industry had merit, they will not represent an absolute certainty and a court of law could pass judgment on other bases or guidelines.

Nevertheless, this approach does provide some framework in which breeders might proceed.

### CONSEQUENCES FOR THE BREEDERS

The concepts of derivation and dependence do not, fortunately, abolish the "breeder's exemption" which is still stated in the 1991 Act. However, "cosmetic" improvement or plagiarism, which could sometimes have allowed the creation of distinct varieties in the sense of the UPOV Convention, will no longer allow the creation of independent varieties. The consequences for the breeders, the farmers and biological diversity more broadly should be positive and will certainly impact the breeder's work.

#### a) Choice of the parents

Breeders should be certain of their legal access and freedom to use all parent materials employed in their breeding programs. They would have to pay more attention to the results of their breeding work when working with protected varieties within the "breeder's exemption".

#### b) Breeding methods

Any conventional breeding method could, in theory, provide an essentially derived

## Essential Derivation and Dependence - Practical Information

variety. Certain methods appear to give a higher risk of developing essentially derived varieties. Among these methods we include:

- natural or induced mutations;
- repeated backcrosses; (discussions still continue on the number of backcrosses which could lead to an essentially derived variety. As shown in the French text of the 1991 Convention, which is of evidence, the authors of the Convention had in mind at least two backcrosses, the word being written in plural. However, it must be noted that the selection pressure exerted after the backcross(es) can have an important effect on the final result).
- selection in an existing variety, for example the choice of clones in a synthetic variety;
- transformation by genetic engineering.

### c) Development of technical information

Conformity thresholds for essential derivation, such as presented above, can be defined in the frame of professional agreement (which would be the solution) or, in a case-by-case basis, in decisions by courts of law. In either case, thresholds will come to exist in the years ahead. To know their freedom to operate in relation to such thresholds, breeders will need:

- a good knowledge of the range of phenotypic, molecular and physiological variability of varieties present in the market;
- to know the phenotypic, molecular and physiological profiles of their genetic material and their experimental varieties, as well as their breeding histories and documentation of legal access.

Breeders will need to employ the tools necessary for assessing such profiles in their research programs. Such tools will not only be used for the protection of intellectual property, but should also promote improvement of breeding efficiency.

### d) Keeping of breeding books

Conformity thresholds only, at least in the zone of uncertainty (orange zone), will not allow a decision on derivation and dependence. In case of litigation, information on parental material and breeding methods will be needed. Thus, breeders will need to maintain clear and accurate breeding records. We encourage breeders to seek competent professional legal advice on the best ways to develop and maintain these important records.

## Essentially Derived Variety

Page 1 of 1

**What is an "Essentially Derived Variety"?**

The concept of essentially derived variety was introduced into the 1991 Act of the UPOV Convention in order to avoid plagiarism through mutation, multiple back-crossing and to fill the gap between Plant Breeder's Rights and patents, gap which was becoming important due to the development of the use of patented genetic traits in genetic engineering.

An essentially derived variety is a variety which is distinct and predominantly derived from a protected initial variety, while retaining the essential characteristics of that initial variety.

As indicated as an example in the UPOV Convention, essentially derived varieties may be obtained by the selection of a natural or induced mutant, or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, back-crossing, or transformation by genetic engineering.

The commercialization of an essentially derived variety needs the authorization of the owner of the rights vested in the initial variety.

The concept of essentially derived variety does not at all abolish the Breeder's Exemption, as free access to protected plant varieties for breeding purposes is maintained. It is not a threat to biodiversity. On the contrary, it favors biodiversity, encouraging breeders developing and marketing original varieties.

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